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POTENTIAL OF *LAETISARIA ARVALIS* FOR THE BIOCONTROL OF *RHIZOCTONIA SOLANI*

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Summary—Bran preparations of the fungus *Laetisaria arvalis* isolates LA-1, OK-60, OK-160, OK-206 and ZH-4 reduced survival and saprophytic growth of *Rhizoctonia solani* (AG-4) in soil. They also prevented damping-off (*R. solani*) of cotton, sugar beet, lettuce and radish in the greenhouse. The six isolates of *L. arvalis* grown on sterile wheat bran moistened with water and incubated for 5–15 days before addition to soil at a rate of 0.5% (w/w), were equally effective in reducing *R. solani* inoculum. In general, 15 day old preparations were as effective as 5 day old preparations. 7 Day old bran preparations of various *L. arvalis* isolates added to pathogen-infested loamy sand soil at a rate of 0.5% (w/w), prevented post-emergence damping-off of cotton, sugar beet, radish and lettuce. Several treatments resulted in seedling emergence and survival similar to those in natural soils not supplemented with *R. solani*. Preparations of *L. arvalis* also prevented damping-off of cotton in three other soils.

INTRODUCTION

In addition to *Trichoderma* and *Gliocladium*, other fungi have the potential for use in biocontrol. These include *Acrophialophora* and *Neocosmophora* (Turhan and Turhan, 1989), *Stilbella* (Singh and Webster, 1973), *Talaromyces* (Marois *et al.*, 1982), *Sporidesmium* (Adams and Ayers, 1982), *Coniothyrium* (Huang, 1980), and *Laetisaria* (Burdsall *et al.*, 1980). For various reasons, these fungi have not been studied to the same extent in biocontrol as have *Trichoderma* or *Gliocladium*.

L. arvalis Burds., a soil-inhabiting basidiomycete originally isolated by Dr M. G. Boosalis of the University of Nebraska, was suggested by Burdsall *et al.* (1980) to be a potential biocontrol fungus. In the greenhouse, the fungus known up to that time as *Corticium*, effectively prevented damping-off of table beet seedlings caused by *Pythium ultimum* (Hoch and Abawi, 1979; Martin *et al.*, 1983, 1986) or by *Phoma betae* (Martin *et al.*, 1984), and crown or brace rot of corn (Sumner and Bell, 1988), damping-off of sugar beet (Odvody *et al.*, 1980), and seedling disease of cotton (Lartey *et al.*, 1991) caused by *Rhizoctonia solani*. In field studies, isolates of *L. arvalis* prevented damping-off of sugar beet (Odvody *et al.*, 1980), peppers (Conway, 1986), and fruit rot of cucumbers (Lewis and Papavizas, 1980) caused by *R. solani*. Southern blight of apple seedlings caused by *Sclerotium rolfsii* was also reduced by *L. arvalis* (Conway, 1986). Reduction in inoculum density of *R. solani* was associated with an increase in population of *L. arvalis* (Odvody *et al.*, 1980; Martin *et al.*, 1983, 1986; Allen *et al.*, 1985; Larsen *et al.*, 1985). In other field tests, preparations of isolates of *L. arvalis* did not reduce damping-off of sugar beet seedlings caused by *R. solani* (Allen *et al.*, 1985; Larsen *et al.*, 1985; Herr, 1988).

Various formulations and delivery systems have been used with *L. arvalis*. The fungus was applied to seed and soil as biomass containing sclerotia and hyphae (Odvody *et al.*, 1980; Martin *et al.*, 1984, 1986; Allen *et al.*, 1985; Lartey *et al.*, 1991), or as a bulk amendment of the fungus grown on solid substrates such as corn leaf meal and sugar beet pulp (Martin *et al.*, 1983), wheat bran (Martin *et al.*, 1984), barley grain (Herr, 1988) and sand corn meal (Lewis and Papavizas, 1980). *L. arvalis* was also added to a fluid-gel and applied to seeds or was used as a dip for fruit tree seedlings (Conway, 1986).

Our purpose was to further evaluate the biocontrol potential of a bran preparation of *L. arvalis* as a basis for possible commercial development of the antagonist. Data are presented on the reduction of inoculum density of *R. solani* and of damping-off of several hosts caused by the pathogen in the greenhouse. A preliminary report has appeared (Lewis *et al.*, 1988).

MATERIALS AND METHODS

Soils and fungal cultures

A loamy sand I [pH 6.4, 0.4% organic matter (OM), 84% sand (S), 8% silt (Si), 8% clay (C)] was used in most experiments. Where indicated, additional soils used included another loamy sand II (pH 5.8, 0.5% OM, 90% S, 5% Si, 5% C), a sandy loam (pH 5.1, 0.2% OM, 77% S, 12% Si, 11% C), and a sandy clay loam (pH 4.5, 3.2% OM, 46% S, 21% Si, 33% C). The soils were screened (<2.0 mm) and maintained at *ca* −30 kPa for 3 weeks before use. Fertilizer (N–P–K; 10–10–10) was added to soils at a rate of 0.05% (w/w) along with inoculum of *R. solani*.

Isolate R-23 of *R. solani* Kühn (AG-4), used in all studies, was maintained on potato dextrose agar (PDA). To study survival and saprophytic growth of *R. solani*, pathogen-infested table beet (*Beta vulgaris* L.) seed was used to simulate natural inoculum of mycelium embedded in organic debris. Beet seed (25 g) was mixed with water (25 ml), placed in 250-ml Erlenmeyer flasks, autoclaved and inoculated with an agar plug (6 mm dia) of *R. solani*. Preparations were kept at 21–23°C for 4 weeks and then air-dried (Papavizas and Lewis, 1986). This inoculum could be kept for several months without appreciable loss in viability. To study damping-off incited by *R. solani*, inoculum was prepared that contained the pathogen grown on millet (*Pennisetum glaucum* L.) seed in place of beet seed.

Six isolates of *L. arvalis* were used; isolates LA-1 and ZH-4 were obtained from M. G. Boosalis (University of Nebraska) and H. H. Burdsall (University of Wisconsin), respectively, and isolates OK-60, OK-160, OK-167 and OK-206 from K. E. Conway (University of Oklahoma). Isolates were maintained on PDA. 1 Week old PDA cultures containing hyphae were comminuted in sterile water (1 plate $50\,\mathrm{ml}^{-1}$ of water) and 5 ml of the suspension were added to 50 g of sterile wheat bran and water (1:1, w/w) in 1-liter Erlenmeyer flasks (Lewis and Papavizas, 1987). Flasks were kept for 3, 5, 7 or 15 days at 21–23°C. The bran in flasks with *L. arvalis* was thoroughly colonized by hyphae of the fungus, but the characteristic brick-red sclerotia of *L. arvalis* were not evident on the bran even after 15 days of growth (Burdsall *et al.*, 1980).

Survival and saprophytic growth of R. solani in soil

Soil (loamy sand I) was supplemented with pathogen-infested table beet seed (1.2 g 200 g soil^{-1}, dry wt equivalent). Concurrently, wheat bran, infested with isolates of *L. arvalis* and incubated for 5 or 15 days was mixed with the soil at a rate of 0.5% (w/w). Soils were maintained at −30 kPa. After 3 weeks of incubation, seed was retrieved from 200 g of soil on a sieve with 1.4 mm openings, washed, and 10 seeds were placed on each of five plates of 2% water agar with antibiotics (Papavizas and Lewis, 1986). The characteristic, branched growth of *R. solani* was detected on plates after 20–24 h at 23–25°C. When needed, nystatin was also added to the water agar to inhibit development of *L. arvalis* (Papavizas *et al.*, 1983). Survival was expressed as a colonization index (CI) of 0–5 based on the extent of hyphal growth on the agar surface from each beet seed: 0 = no observable hyphae of *R. solani* from seed; 1 = several threads or strands of hyphae; 2,3,4,5 = hyphae on 25, 50, 75% and all of the agar surface surrounding the beet seed, respectively (Lewis *et al.*, 1991).

To determine the saprophytic growth of *R. solani* from infested beet seed into soil, autoclaved, non-infested beet seed (1.2 g) was added to the sieved soil portions (200 g) as a bait to trap hyphae of the pathogen which grew into the soil (Papavizas and Lewis, 1986). Soils were incubated 4 days, beet seed was retrieved, washed, plated, and observed as above. The results, expressed as a CI, reflected the growth or saprophytic activity of *R. solani* from infested beet seed into the soil. In all instances, controls consisted of pathogen-infested soils amended with bran not containing *L. arvalis*.

Effect of L. arvalis on damping-off

1 kg (dry wt equivalent) portions of natural soils were infested with isolate R-23 of *R. solani* grown on millet seed at a rate of 0.015% (w/w) and moistened. Bran preparations of isolates of *L. arvalis* (grown for 3, 7 or 15 days) were added 7 days later to soil batches at a rate of 0.5% (w/w). Soils were placed in plastic flats (18 × 12 × 6.5 cm), kept for an additional week, and planted with metalaxyl-treated seed (0.4 g a.i. kg^{-1} seed) of cotton (*Gossypium hirsutum* L. Stoneville 213), sugar beet (*Beta vulgaris*

Table 1. Survival and saprophytic growth of *R. solani* (R-23) in soil with 5 and 15 day old bran preparations of six isolates of *L. arvalis**

Isolate	Survival of R-23 in infested beet seed (CI)†		Saprophytic growth of R-23 from infested beet seed into soil (CI)	
	5 day old inoculum	15 day old inoculum	5 day old inoculum	15 day old inoculum
Control (R-23, bran)‡	4.5 a§	4.5 a	4.5 a	3.3 abc
LA-1	2.0 c	2.8 c	1.0 d	2.4 d
OK-60	2.6 c	3.8 b	2.6 bc	2.6 cd
OK-160	2.3 c	3.1 bc	1.2 d	3.7 a
OK-167	2.8 bc	3.7 bc	2.2 c	2.9 bcd
OK-206	3.6 b	3.9 ab	3.1 b	3.4 ab
ZH-4	2.0 c	2.6 c	1.3 d	2.0 d

*Assay performed 3 weeks after concurrent addition to soil of bran preparations and pathogen-infested beet seed.
†Colonization index (CI) indicates growth of *R. solani* on agar surface (see text).
‡Bran preparations, containing mycelia of isolates of *L. arvalis* grown for 5 and 15 days, added to a loamy sand at a rate of 0.5% (w/w). Preparations added to *R. solani*-infested control soil contained bran with no *L. arvalis*.
§Values in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leqslant 0.05$).

Table 2. Effect of age of bran preparations of isolates of *L. arvalis* on damping-off of cotton and sugar beet caused by *R. solani* (R-23) in loamy sand I

Isolate	Inoculum age (days)	Plant stand (%) at 3 weeks	
		Cotton	Sugar beet
Control (no R-23)		82 a*	63 a
Control (R-23)		25 d	20 de
Control (R-23, bran)†		18 d	6 e
LA-1	0	36 cd	39 bc
	3	70 ab	49 ab
	7	66 ab	60 a
	15	79 a	51 ab
ZH-4	0	21 d	30 cd
	3	75 ab	39 bc
	7	56 bc	49 ab
	15	66 ab	60 a

*Values in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leqslant 0.05$).

†Bran preparations, containing mycelium of isolates of *L. arvalis* grown for 0, 3, 7 or 14 days, added to soils at a rate of 0.5% (w/w). Preparations added to *R. solani*-infested control soil contained bran with no *L. arvalis* biomass.

L. USH-20), radish (*Raphanus sativus* L. Scarlet Globe) and lettuce (*Lactuca sativa* L. Salad Bowl). Flats were planted with three rows of five seeds per row of cotton and with three rows of eight seeds per row of sugar beet, radish and lettuce, and were maintained in the greenhouse at 21–23°C. Emergence and plant survival were determined 1, 2 and 3 weeks after planting.

Statistical analysis

Each experiment with appropriate controls was performed twice with five replications. The data presented are the results of the second experiment. An analysis of variance (ANOVA) indicated no difference between the experiments. Significant differences among treatments were determined with Duncan's multiple range test. Percentage values were analyzed after arcsin-transformation of the raw data.

RESULTS

Survival and saprophytic growth of R. solani in soil

Bran preparations of *L. arvalis* effectively reduced the inoculum density of *R. solani* in soil (Table 1). 5 Day old bran preparations of each of the six isolates tested and 15 day old preparations of five isolates significantly reduced survival of *R. solani* in beet seed. The growth of *R. solani* from beet seed into soil (saprophytic activity) was also prevented by all 5 day old preparations, but only by two of the 15 day old preparations (LA-1, ZH-4). However, the magnitude of reduction of both the pathogen activity and its survival by the 15 day old preparations was not as great as that of the 5 day old preparations. Bran preparations of isolates LA-1 and ZH-4 grown for either period were the most effective in reducing pathogen inoculum.

Effect of L. arvalis on damping-off

Observation of the weekly stand counts indicated that most of the cotton, sugar beet, radish and lettuce seed had emerged within 10–12 days of planting. When damping-off of seedlings occurred, it progressed rapidly. Consequently, >75% of the disease incidence was considered to be due to post-emergence damping-off.

3, 7 and 15 day old bran preparations of *L. arvalis* isolates LA-1 and ZH-4 were evaluated for their

Table 3. Stand of cotton in three soils infested with *R. solani* (R-23) amended with bran preparations of isolates of *L. arvalis*

Isolate	Plant stand (%) at 3 weeks in:		
	Sandy loam	Loamy sand II	Sandy clay loam
Control (no R-23)	90 a*	88 a	68 b
Control (R-23)	20 c	10 c	21 c
Control (R-23, bran)†	10 c	10 c	12 c
LA-1	85 ab	80 ab	84 a
OK-60	74 b	70 b	80 a
OK-160	74 b	68 b	73 ab
OK-167	80 ab	75 ab	81 a
OK-206	70 b	65 b	78 ab
ZH-4	78 ab	74 ab	73 ab

*Values in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leqslant 0.05$).

†Bran preparations, containing mycelium of isolates of *L. arvalis* grown for 7 days, added to soils at a rate of 0.5% (w/w). Preparations added to *R. solani*-infested control soil contained bran without *L. arvalis* biomass.

Table 4. Effect of bran preparations of isolates of *L. arvalis* on damping-off of radish and lettuce caused by *R. solani* (R-23) in loamy sand I

Isolate	Plant stand (%) at 3 weeks	
	Radish	Lettuce
Control (no R-23)	76 a*	50 abc
Control (R-23)	10 e	8 d
Control (R-23, bran)†	0 e	0 e
LA-1	70 ab	58 ab
OK-60	59 bc	62 a
OK-160	44 d	36 c
OK-167	49 cd	43 bc
OK-206	69 ab	65 a
ZH-4	63 ab	65 a

*Values in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leqslant 0.05$).

†Bran preparations, containing mycelium of isolates of *L. arvalis* grown for 7 days, added to soils at a rate of 0.5% (w/w). Preparations added to *R. solani*-infested control soil contained bran without *L. arvalis* biomass.

ability to prevent damping-off of cotton and sugar beet in a loamy sand in the greenhouse (Table 2). In general, both isolates at each inoculum age were equally effective in preventing the disease ($P < 0.05$) and gave stands similar to those in control soil not infested with the pathogen. Disease on cotton was not prevented in soil amended with freshly-inoculated bran (0-day incubation). However, there was a slight but significant effect of this preparation on sugar beet stand (Table 2).

Bran preparations of isolates of *L. arvalis* also prevented damping-off of cotton in various types of soil (Table 3). 7 Day old preparations of each of the six isolates studied were effective against Rhizoctonia damping-off of cotton in a sandy loam, sandy clay loam and another sample of a loamy sand. There was no difference in effectiveness among isolates in each of the soils. However, in sandy loam and loamy sand II, bran preparations of isolates LA-1, OK-167 and ZH-4 gave stands similar to those in control soils not infested with the pathogen. In sandy clay loam, which was a heavier soil than the other two, preparations of OK-160, OK-206 and ZH-4 gave stands similar to that of the control soil, but preparations of LA-1, OK-60 and OK-167 resulted in stands greater than that of the control soil (Table 3).

The ability of isolates of *L. arvalis* to prevent damping-off on crops other than cotton and sugar beet was investigated by studying the effect of 7 day old bran preparations of the six isolates on damping-off of radish and lettuce in a loamy sand (Table 4). All isolates prevented damping-off of radish and lettuce, but, unlike their biocontrol ability with cotton and sugar beet, the isolates varied in their effectiveness. For example, isolates OK-160 and OK-167 were less effective in disease suppression than the other four isolates.

DISCUSSION

The data indicate the biocontrol potential of *L. arvalis* for effectiveness against damping-off diseases of several crops caused by *R. solani* and support the results of earlier investigations. The investigation of several experimental variables in our work may contribute to a greater understanding of the fungus for additional studies leading to possible commercialization.

The most significant aspect of this research was the use of a solid, bulk preparation of the biocontrol fungus incubated for a short time before its application to a natural soil at a relatively low rate. The effective system used, wheat bran: water (1:1, w/w) to which inoculum of *L. arvalis* was added and allowed to grow for 3–7 days before addition to soil at a rate of 0.5%, compared favorably with systems previously used. For example, in other reports, wheat bran infested with *L. arvalis* was added to soil after 2–4 weeks of growth at rates of 5–10% (Hoch and Abawi, 1979; Martin *et al.*, 1984). Martin *et al.* (1983), however, questioned the use of large amounts of amendment. Our rationale for using young, actively-growing cultures (3–5 days) was based on previous observations in which young, actively-growing cultures of *Trichoderma* spp and *G. virens* on bran (germlings) reduced inoculum density of *R. solani* and damping-off better than older preparations in which fungi had formed conidia (Lewis and Papavizas, 1987). Our results indicated that 5 day old inoculum of *L. arvalis* on bran was as effective in disease reduction as 15 day old inoculum.

The manner in which biocontrol preparations are developed and formulated as well as the amounts applied greatly affect their commercial potential. Concerns with growth media were addressed by Odvody *et al.* (1980), who indicated that *L. arvalis* was more effective in controlling *R. solani* when added to soil in a sugar beet pulp mixture than when introduced as sclerotia alone. Although the work of Martin *et al.* (1984) and our work have shown wheat bran to be an effective food base and carrier, culturing *L. arvalis* on materials such as bran and grains (Herr, 1988) may not be the best method for inoculum preparation. Since facilities are already in place, liquid (aerobic) fermentation may be more desirable. It has already been shown that biomass of mycelia and sclerotia of *L. arvalis* can develop on potato dextrose broth (Martin *et al.*, 1984; Allen *et al.*, 1985; Lartey *et al.*, 1991). We have produced effective biomass of *L. arvalis* on a molasses–brewer's yeast medium which can be formulated into alginate prill (Lewis and Papavizas, 1985). These results suggest the possibility for growing the antagonist in large-scale liquid (aerobic) fermentation on readily-available, inexpensive substrates similar to those used for production of biomass of *Trichoderma* spp and *G. virens* (Papavizas *et al.*, 1984).

Another desirable characteristic in a biocontrol fungus is the ability of several isolates of the fungus to control a disease on several crops and to be effective in different soil types. For example, each of the six isolates we used reduced the inoculum density of *R. solani* and prevented its spread in soil (Table 1). Odvody *et al.* (1980) and Martin *et al.* (1984, 1986) reported that the biocontrol fungus reduced the population density of *R. solani* as well as that of *P. betae* and *P. ultimum*. Our results also show that isolates of *L. arvalis* prevented damping-off on a variety of crops, an attribute also demonstrated by Hoch and Abawi (1979), Odvody *et al.* (1980), Conway (1986), Martin *et al.* (1986) and Lartey *et al.* (1991). Although all these studies were performed in various soils, to our knowledge, our data are the first to demonstrate the biocontrol ability of several isolates of *L. arvalis* in various soil types at one time (Table 3). The results suggest the potential for the fungus to be effective in various soils of different texture, organic matter content and pH. In a limited study (Hoch and Abawi, 1979), the biocontrol induced by *L. arvalis* was investigated at various temperatures and moistures. However, the effects of edaphic factors on activity of the fungus remain to be elucidated. In addition, in order to consider the antagonist for commercial biocontrol, its survival, multiplication and survival in production systems require serious attention. Future studies should include innovative approaches for growth of the antagonist and its formulation, adequate field testing, ecological studies and its mechanism of action.

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